

# Epithelial and Mesenchymal Features of Pancreatic Ductal Adenocarcinoma Cell Lines in 2- and 3-Dimensional Cultures

Yuuki Shichi<sup>1</sup>, Fujiya Gomi<sup>1</sup>, Norihiko Sasaki<sup>2</sup>, Keisuke Nonaka<sup>1</sup>, Tomio Arai<sup>3</sup>, Toshiyuki Ishiwata<sup>1\*</sup>

## Document S1: Supplementary materials and methods

### *Search criteria for human pancreatic cancer cell lines in the Cellosaurus*

A search for "pancreatic cancer" in the Cellosaurus revealed 629 cell lines, and narrowing down the species to *Homo sapiens* resulted in 527 cell lines. Data on cancers such as invasive breast carcinoma of no special type, breast ductal carcinoma, ductal cell of pancreas, high-grade ovarian serous adenocarcinoma, esophageal squamous cell carcinoma, and stellate cell were mixed; therefore, they were excluded (417 cell lines). The data obtained were then segregated to PDAC (377 cell lines) and other pancreatic cancer (40 cell lines) cell lines.

### *Cell culture*

Human PDAC cell line PK-1 and MIA PaCa-2 cells were obtained from the Cell Resource Center for Biomedical Research, Institute of Development, Aging and Cancer, Tohoku University (Sendai, Japan). Cells were grown in a growth medium (RPMI-1640 medium containing 10% fetal bovine serum) at 37 °C in a humidified 5% CO<sub>2</sub> atmosphere. To form spheres, cells ( $3 \times 10^3$  cells/well) were plated in 96-well ultra-low attachment plates (Thermo Fisher Scientific, Waltham, MA, USA) with a growth medium. After 7 days, the spheres were photographed using a phase-contrast microscope (Eclipse TS-100, Nikon, Tokyo, Japan). Spheres were then aspirated using micropipettes and used for further experiments.

### *Scanning electron microscopy (SEM)*

Adherent PK-1 and MIA PaCa-2 cells were fixed for 30min, and PK-1 and MIA PaCa-2 spheres were fixed overnight with 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) at 4°C. Then, the glutaraldehyde solution was removed, and the cells were washed with phosphate-buffered saline. The PDAC cells were post-fixed with osmium tetroxide for 30 min. After complete dehydration via a graded ethanol series, samples were suspended in 100% ethanol, air-dried, and coated with a platinum layer using an MSP-1S sputter coater (Shinku Device, Ibaraki,

Japan). Cells were photographed with a Phenom Pro desktop scanning electron microscope using secondary electrons and reflected electrons for attached cells and spheres, respectively (Thermo Fisher Scientific).

#### *Immunocytochemical analysis of cell blocks for spheres*

The spheres were collected using a micropipette under a microscope and then fixed in 10% neutral-buffered formalin for 3 h. Formalin was removed using a micropipette, and embedded in paraffin. Serial sections of the cell blocks (4- $\mu$ m thickness) were immunostained using the labeled streptavidin-biotin method. The primary antibodies used in immunocytochemical staining were as follows: mouse monoclonal anti-E-cadherin antibody (M106) from Takara Bio (Shiga, Japan) and mouse monoclonal anti-vimentin antibody (422101) from Nichirei Biosciences Inc. (Tokyo, Japan). Sections were treated with 0.03% H<sub>2</sub>O<sub>2</sub> in 33% methanol at room temperature for 30 min to block endogenous peroxidase before antigen retrieval treatment. The reaction to each antigen was visualized by adding 3,3'-diaminobenzidine tetrahydrochloride (DAB) and counterstaining with hematoxylin. The images were taken with an upright microscope (BX43; Olympus, Tokyo, Japan).

**Table S1.** Primer list <sup>(15, 34)</sup>

<i>Gene</i>	Forward primer	Reverse primer
<i>E-cadherin</i>	CCAGTGAACAACGATGGCATT	TGCTGCTTGGCCTCAAAAT
<i>Vimentin</i>	TCCAAACTTTTCCTCCCTGAAC	GGGTATCAACCAGAGGGAGTGA
<i><math>\beta</math>-ACTIN</i>	GGTCATCACCATTGGCAATGAG	TACAGGTCTTTGCGGATGTCC